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ROLE OF AVIAN TRIGEMINAL SENSORY SYSTEM IN DETECTING CONIFERYL BENZOATE, A PLANT ALLELOCHEMICAL

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Abstract—Coniferyl benzoate, a secondary metabolite found in quaking aspen (*Populus tremuloides*) and other plants, is an avian feeding deterrent of ecological and potential commercial importance. This study was conducted to determine if coniferyl benzoate is a trigeminal stimulant for birds and to ascertain if trigeminal chemoreception of coniferyl benzoate can mediate avian feeding behavior. Five European starlings (*Sturnus vulgaris*) with bilateral nerve cuts (ophthalmic branch of the trigeminal nerve) and four starlings that had sham surgeries were fed a commercial diet treated with coniferyl benzoate. Birds receiving bilateral nerve cuts ate significantly more feed than intact birds, indicating trigeminal detection of coniferyl benzoate and trigeminal mediation of feeding behavior. In the past, trigeminal chemoreception has not been recognized as important in the detection of plant secondary metabolites despite the irritant or astringent properties of a number of them.

Key Words—European starlings, *Sturnus vulgaris*, ruffed grouse, *Bonasa umbellus*, phenylpropanoid, feeding deterrent, chemical senses.

INTRODUCTION

Anatomical and behavioral evidence that the chemical senses (i.e., taste, smell, and trigeminal) are sufficiently evolved in birds to permit discrimination among

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chemical cues has existed for some time (Wenzel, 1973). However, research on the diverse ways birds make use of their chemical senses (e.g., Baltharzart and Schoffeniels, 1979; Clark and Mason, 1985, 1988; Wenzel, 1986; Waldvogel, 1987) and the importance of avian chemoreception in the management of pest species (e.g., Mason and Silver, 1983; Mason et al., 1989; Mason, 1989) are just coming to the forefront.

Understanding how birds detect plant secondary metabolites could provide additional insights into avian ecology and the development of avian repellents. Plant secondary metabolites can influence avian food selection (Cook et al., 1971; Remington and Braun, 1985; Greig-Smith and Wilson, 1985; Jakubas et al., 1989), nestling parasite loads (Clark and Mason, 1988), and possibly, population densities (Jakubas and Gullion, 1991). In an applied context, the chemical structures of aversive plant secondary metabolites can serve as chemical models for developing avian repellents (Crocker and Perry, 1990; Jakubas et al., 1991). In this regard, phenylpropanoids related to cinnamic acid and coniferyl alcohol may be both ecologically and commercially important (Jakubas et al., 1989; Crocker and Perry, 1990; Jakubas et al., 1991). For example, coniferyl benzoate, an ester of coniferyl alcohol, is a feeding deterrent for ruffed grouse (*Bonasa umbellus*) (Jakubas and Gullion, 1990), European starlings (*Sturnus vulgaris*) (Jakubas et al., 1991), and possibly other passerines (Jakubas et al., 1989). Coniferyl benzoate naturally occurs in quaking aspen (*Populus tremuloides*); in gum benzoin Siam, from *Styrax tonkinensis*; and in jasmine oil (Freudenberg and Bittern, 1950; Adamson, 1972; Kato, 1984; Jakubas et al., 1989). Ecologically, it appears to be important in mediating the feeding behavior of ruffed grouse when they select quaking aspen flower buds (Jakubas et al., 1989; Jakubas and Gullion, 1990, 1991). It has been proposed that the irritant property of coniferyl benzoate may be one of the primary sensory cues used by ruffed grouse when selecting aspen buds (Jakubas, 1989).

Chemical irritation, such as from spicy foods or noxious fumes (e.g., ammonia) are sensations commonly attributed to trigeminal chemoreception (Silver, 1987). Trigeminal sensations (the common chemical sense) occur from stimulation of free nerve endings in the epithelium or mucosa and include the sensations of touch, pain, temperature, and proprioception (movement) (Silver, 1987). In most terrestrial vertebrates, the trigeminal (Vth) nerve innervates the epithelia of the head region, including intranasal, intraoral, and corneal surfaces (Silver, 1987). In the birds, the trigeminal nerve is principally divided into three branches, the ophthalmic (upper mandible and eye), maxillary (above eye and between mandibles), and mandibular (lower jaw and muscles) (Breazile and Yasuda, 1979).

Coniferyl benzoate was proposed to be a trigeminal stimulant because of the burning sensation it produces when ingested or rubbed on the eyelids (Jakubas, 1989, and unpublished data). In addition, coniferyl benzoate often will cause an eczematous reaction when applied to the skin (Hjorth, 1961; Kato, 1984). Although coniferyl benzoate is a human irritant, this may not mean that birds perceive it similarly. For example, capsaicin, the pungent chemical found in peppers of the Capsicum family, is a strong trigeminal irritant to mammals but not to birds (Mason and Maruniak, 1983; Szolcsanyi et al., 1986). Confirmation that birds use trigeminal chemoreception to sense coniferyl benzoate, may help explain the repellency of analogous compounds (see Jakubas et al., 1991).

The objectives of this study were to (1) determine if coniferyl benzoate is a trigeminal stimulant for birds and (2) ascertain if trigeminal chemoreception of coniferyl benzoate can mediate avian feeding behavior. European starlings were chosen for this study because they have good chemosensory abilities (Clark and Mason, 1987; Espaillat and Mason, 1990) and are similar to ruffed grouse in their sensitivity to dietary coniferyl benzoate (see Jakubas et al., 1991; Jakubas and Gullion, 1990). In addition, anesthesia and surgical techniques for denervation of chemosensory systems in starlings are well documented (Mason and Silver, 1983; Clark and Mason, 1987; Mason et al., 1989).

METHODS AND MATERIALS

Diet Preparation. Coniferyl benzoate was obtained by continuous liquid–liquid extraction of benzoin Siam tears #3 (Alfred L. Wolff, Paris, France) and purified by crystallization following the procedures in Jakubas et al. (1991). Coniferyl benzoate was added to the test feed (5:1 mixture of Chick Starter and AVN Canary/Finch diet; Purina Mills Inc., St. Louis, Missouri) by dissolving a known quantity of crystalline material in diethyl ether, thoroughly mixing the solution with the feed, and evaporating the ether under a hood. The level of coniferyl benzoate applied to the feed (3.2% w/w) corresponds to levels in aspen flower buds that are generally not fed on by ruffed grouse (Jakubas and Gullion, 1990, 1991). Treated feed was stored under nitrogen, at −17°C, to prevent decomposition of coniferyl benzoate. The control feed was prepared by mixing the test feed with diethyl ether and evaporating the ether as described above.

Feeding Trials. Twelve European starlings were captured using a funnel trap in Philadelphia, Pennsylvania, during early April and transferred to indoor facilities three weeks prior to testing. The birds were individually caged (61 × 36 × 41-cm cages) and housed under constant temperature conditions (approx. 22°C), with a 11:13 hr light–dark cycle. Water and feed were provided ad libitum. The maintenance diet was a 5:1 mixture of Chick Starter and AVN Canary/Finch diet, to which oyster shell grit (United Volunteer Aviaries, Nashville, Tennessee) was added.

Seven days before surgery, birds were conditioned for five days to a food-deprivation routine. During this time, birds were deprived of their normal diet overnight. The following morning they were given 10 g of control feed approximately 2 hr after light onset. After 2 hr, consumption was measured and the maintenance diet returned to the cages for the remaining hours of light. Tap water was available ad libitum throughout the conditioning period and subsequent feeding trials.

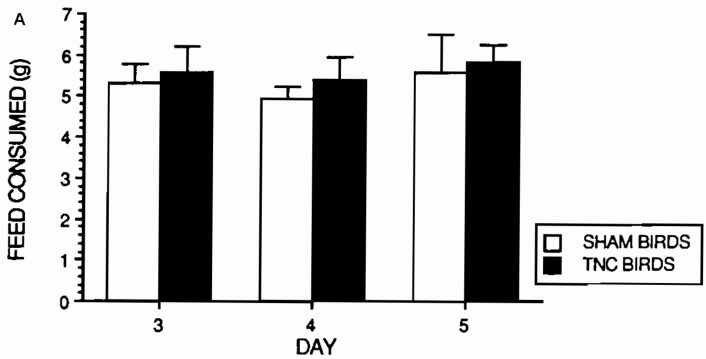
On day 6 (one day prior to surgery), starlings were randomly assigned to receive either bilateral trigeminal nerve cuts (TNC) or sham surgeries and were food-deprived overnight. For TNC, birds were anesthetized by intraperitoneal injection of chloropent (4 ml/kg body wt) and placed in a head holder. To avoid disturbing the olfactory nerve, the ophthalmic branches of the trigeminal nerve were exposed and cut at the junction of the nervus ophthalmicus: ramus medialis and ramus nasales (see Breazile and Yasuda, 1979). Cut nerve ends were reflected to impede regeneration. The resulting cavity was packed with gelfoam and the skin closed with cyanoacrylate glue. Identical procedures were followed for sham surgeries except the trigeminal nerve was not cut. Five of six birds survived the TNC surgery, and four of six the sham surgery.

Starlings were allowed to recover from surgery for two days, after which they were given 2-hr feeding trials on each of seven days. Starlings were presented control feed on days 3–5 to evaluate their postsurgery condition. On days 6–9, birds were given the treatment diet containing 3.2% coniferyl benzoate.

Consumption of the control diet during the three days preceding and following surgery was analyzed in a three-factor analysis of variance (ANOVA) with repeated measures over days. Similarly, consumption of the treated diet was assessed in a two-factor ANOVA with repeated measures over days. Tukey's Honestly Significant Difference tests (Winer, 1962, p. 198) were used to isolate significant ($P < 0.05$) differences among means. Birds were euthanized by an overdose, via intraperitoneal injection, of sodium pentobarbitol. Nerve cuts were confirmed by necropsy.

RESULTS

Two-hour consumption of the control diet did not differ before or after surgery ($P > 0.25$). Prior to surgery, birds that were subsequently given TNC consumed 5.31 ± 0.25 g of control feed, while sham-operated birds ate 5.21 ± 0.31 g of feed. Following surgery, TNC birds consumed 5.59 ± 0.29 g of control feed and sham-operated birds ate 5.27 ± 0.34 g. However, TNC birds consumed significantly more of the 3.2% coniferyl benzoate diet than sham birds ($F = 22.8$; 1,7 df; $P < 0.01$) (Figure 1). Mean consumption of the 3.2% coniferyl benzoate diet over the four days of the trial was 0.99 ± 0.14 g for



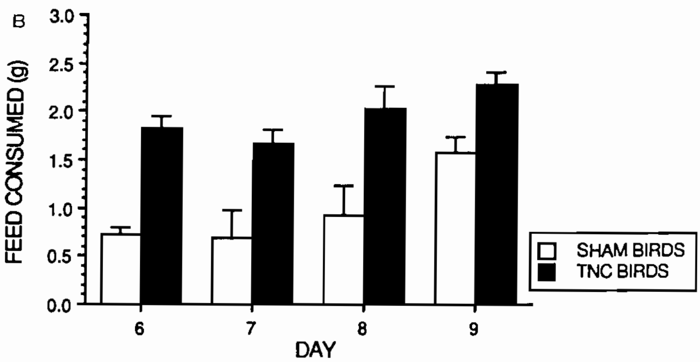


FIG. 1. Daily mean consumption (with standard error bars) during 2-hr feeding trials of (A) control feed and (B) feed treated with coniferyl benzoate (3.2% w/w) for starlings that received bilateral trigeminal nerve cuts (TNC) or sham surgeries.

sham-operated birds and 1.95 $\pm$ 0.09 g for the TNC birds. Over days, consumption differed (F = 10.3; 3,21 df; $P < 0.01$), and post-hoc tests revealed that for both TNC and sham birds, consumption on day 9 was significantly higher than consumption on day 6 (Figure 1). Necropsies on the TNC starlings did not reveal any regrowth of the sectioned trigeminal nerves.

DISCUSSION

Birds with bilateral sections of the ophthalmic branch of the trigeminal nerve (TNC) ate more feed treated with coniferyl benzoate than did sham birds. However, the groups did not differ in consumption of the control feed, suggesting that birds receiving TNC were less sensitive to coniferyl benzoate than intact birds and that trigeminal chemoreception was involved in detection of this compound. These findings agree with other studies indicating that nasal–trigeminal chemoreception can mediate avian feeding behavior and that chemical repellency in birds, in some cases, may be associated with compounds that elicit a trigeminal response (Mason and Silver, 1983; Mason et al., 1989; but see Mason and Maruniak, 1983).

Trigeminal chemoreception has not been recognized in the past as being important to the perception of plant secondary metabolites by animals. For example, Chapman and Blaney (1979) recognized that the chemical senses are commonly used to perceive plant secondary metabolites; however, they incorrectly assumed that chemical irritation was not mediated by the chemical senses and virtually disregarded the importance of trigeminal chemoreception in this early review article.

An example of how trigeminal chemoreception may affect the feeding behavior of animals in the field can be illustrated with ruffed grouse and their selection of aspen buds. Typical avian trigeminal innervation (see Breazile and Yasuda, 1979) should enable ruffed grouse to sense coniferyl benzoate as it manipulates aspen buds with its beak when feeding. Consequently, the level of coniferyl benzoate in the copious external resin of the bud might serve as the initial cue to the bud's suitability. Trigeminal chemoreception (and other chemical senses) may be especially important to ruffed grouse when selecting aspen buds, due to a lack of visible patterns (e.g., color, size, outer resin, and UV absorbance) that could be used to distinguish buds having high levels of coniferyl benzoate or nutrients (Jakubas, 1989, and unpublished data). Indeed, preliminary data indicate that grouse do not feed in trees having buds that produced an oral burning sensation (human perception) (Jakubas, 1989). Given the unspecialized nature of trigeminal receptors (i.e., free nerve endings), the use of trigeminal chemoreception by starlings to detect coniferyl benzoate, by inference, supports the concept that ruffed grouse may use trigeminal chemoreception to detect differential levels of coniferyl benzoate. Differences in trigeminal chemoreception do exist among species; however, many irritants are perceived similarly (Szolcsanyi et al., 1986; Harti et al., 1989). Since trigeminal chemoreception is apparently involved in detecting coniferyl benzoate in species (i.e., humans and starlings) from two animal classes, it seems unlikely that this compound would not elicit a similar response in grouse.

Although ophthalmic nerve sections in starlings appear to have decreased their aversion to coniferyl benzoate-treated feed, it is obvious that TNC birds still found it less suitable than control feed (Figure 1). One explanation for the difference in consumption of control (5.59 ± 0.29 g) and treated feed (1.95 ± 0.09 g) among TNC birds may be the incomplete denervation of the trigeminal (Vth) nerve. Both the mandibular and maxillary branches of the nerve were left intact. Complete denervation was not attempted because it would likely have resulted in prolonged aphagia and lack of responsiveness to food (see Zeigler and Karten, 1973a,b). Alternatively, the data suggest that taste and/or olfaction may have been involved in detection of coniferyl benzoate. Passerine species possess functional olfactory (Clark and Mason, 1989) and taste (Espaillat and Mason, 1990) capabilities, and either or both may have influenced the starling's response.

The increase in consumption of feed treated with coniferyl benzoate over time (Figure 1) may indicate that the birds habituated to the effects (sensory or postingestional) of coniferyl benzoate. Whether habituation to this compound occurs in field situations remains open to question. Factors such as increased toxicity, due to higher consumption of coniferyl benzoate or synergistic reactions with other phenolic compounds, may reinforce avoidance of coniferyl benzoate in field situations. We speculate that toxicity was not a significant factor in the laboratory, since the quantity of coniferyl benzoate consumed during the 2-hr feeding trials was relatively small and may have not been sufficient to invoke a toxic response.

Taste and olfaction are frequently cited as important in animal food selection (e.g., Arnold and Hill, 1972) and in the detection of plant secondary metabolites (Chapman and Blaney, 1979). However, based on our study and earlier work indicating that nasal–trigeminal chemoreception can mediate feeding behavior (e.g., Mason and Silver, 1983; Mason et al., 1989), we propose that trigeminal chemoreception may be important in the detection of many of the common secondary metabolites that have irritant or astringent properties. Compounds with these properties include: tannins (astringent), diphenols (irritants), phenylpropanoids (irritants), and diterpenoid esters (irritants) among others (Robinson, 1983). Trigeminal sensations elicited by these compounds may simply serve as a cue for avoidance or may significantly contribute to the compound's repellency.

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